# Activation of Piezo 1 regulates the IL6 signaling pathway in human osteocytes

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**Disclosures:**

INTRODUCTION: Mechanically sensitive osteocytes function as critical regulators of bone homeostasis. Using a physiological human cell-based 3D *in vitro* model of bone [1] to define the osteocyte response to mechanical load we found that loading of osteocytes results in 16-fold upregulation of Piezo1 [2]. In addition, treatment of osteocytes with the mechanical load mimic, Yoda1 upregulates expression of IL6, a cytokine that drives inflammation and bone resorption [2] and has been implicated in the onset of post-traumatic osteoarthritis (PTOA) [3]. Both Yoda1 and mechanical load activate Piezo1 to promote bone formation, deletion of Piezo1 in osteoblasts and osteocytes decreases bone mass and bone strength in mice and SNPs in the Piezo1 locus are associated with low bone mineral density and increased fracture risk [4,5]. This study investigated the effect of IL6 and Yoda1 on human osteocytes, to reveal mechanisms underlying interactions between mechanical load and inflammation implicated in osteoarthritis and fracture healing.

METHODS: Human Y201 MSC cells [6,7] were embedded at a density of 0.025 x $10^6$ cell/gel in type I collagen gels and differentiated to osteocytes in osteogenic media for 13-days. Cells were treated with IL6 (5ng/ml)/sIL6r (40ng/ml), Yoda1 (5μM) [8] or a combination of IL6/sIL6r/Yoda1 for 2- and 24-hours (n=6-10); vehicle treated cells served as controls (n=4-6). RNA was extracted and converted to cDNA prior to RT-qPCR and relative quantification using the ΔΔCt method. cDNA from control and IL6+Yoda1 24-hour treated cells was also analyzed using the human IL6/STAT3 Signaling Plus RT² Profiler PCR Array (Qiagen; regulated >1.5-fold); 7 genes were chosen for validation in duplicate cultures treated with vehicle, IL6, Yoda1 or a combination of IL6+Yoda1. Media was analyzed for proinflammatory cytokines using a multiplex panel ELISA (MSD). Data was analyzed by ANOVA (Minitab).

RESULTS: **IL6 RT-qPCR:** Yoda1 treatment for 2-hrs resulted in a 103-fold increase in IL6 mRNA ($p<0.001$ vs control). Treatment with IL6/sIL6r for 2-hrs resulted in a 7-fold increase in IL6 mRNA ($p=0.009$) whereas the combination of Yoda1 and IL6 resulted in a 5.5-fold increase ($p=0.019$ vs control) which was significantly lower than levels induced by Yoda1 alone ($p=0.001$ vs Yoda1). **IL6/STAT3 PCR array**: IL6/sIL6r + Yoda1 resulted in upregulation of 19 and down regulation of 13 genes involved in the IL6/STAT3 pathway. These included genes upstream of IL6/STAT3 (e.g MAP2K1 4.3-fold; MAPK8 2-fold; SRC 2-fold), cytokines (e.g IL11 4.9-fold; IL1B 8.7-fold; IL6 7.5-fold; IL22 -12.7-fold; TNFSF10 -8.8-fold; CSF3 -3.6-fold; CCL2 -3.9-fold), STAT3 activators (e.g EGFR 3.1-fold), cell surface receptors (e.g TNFRSF10B 2.1-fold; CD4 -4-fold), target genes (e.g CEBPD 2.7-fold; CXCL8 -2.4-fold), genes downstream of IL6/STAT3 (e.g PIM1 2.5-fold; STAT3 1.6-fold; HGF -6.2-fold), and genes involved in the NFkB signaling pathway (e.g RELA 1.7-fold; TLR4 1.9-fold). **PCR array validation:** 7 of the upregulated genes were chosen for validation by RT-qPCR (figure 1). Upregulation of IL6, PIM1, IL1B, IL11, and MAP2K1 by IL6+Yoda1 were confirmed by RT-qPCR. Yoda1 alone caused the greatest increase in the expression of MAP2K1 (8.9-fold, $p<0.001$), IL11 (7.5-fold $p<0.001$), EGFR (13.9-fold, $p<0.001$), and CEBPD (28.4-fold, $p<0.001$). **Multiplex ELISA:** Treatment of osteocytes with Yoda1 for 2-hrs resulted in a small increase in mean IL4 release (1.5-fold, $p=0.107$) and 6.3-fold increase in IL6 release ($p=0.005$). IL6/sIL6r treatment for 2-hrs resulted in an increase in the release of IFN-γ (4.5-fold, $p<0.001$), IL2 (3.8-fold, $p<0.001$), IL1β (2.6-fold, $p<0.001$), IL13 (2.4-fold, $p=0.009$), IL12p70 (3.4-fold, $p=0.001$), TNF-α (2.8-fold, $p=0.001$), IL10 (3.8-fold, $p<0.001$), and IL4 (3.8-fold, $p<0.001$). The combined treatment of Yoda1 and IL6/sIL6r prevented some of the IL6 induced increase in IFN-γ (1.3-fold, $p<0.001$), IL10 (1.4-fold, $p=0.034$), and IL4 (1.4-fold, $p=0.025$) but levels remained significantly higher than control levels. Sustained treatment with Yoda1 for 24-hrs resulted in an increased release of IFN-γ (1.5-fold, $p<0.001$), IL12p70 (2.1-fold, $p<0.001$), IL6 (1.4-fold, $p=0.001$), and IL4 (7-fold, $p<0.001$) and an increase in the mean level of IL10 (1.3-fold, $p=0.09$) and TNFα (1.3-fold, $p=0.147$). In contrast, IL6/sIL6r treatment for 24-hrs only increased release of IL4 (1.3-fold, $p=0.012$) and reduced mean levels of IL13 (1.3-fold, $p=0.057$). The combined treatment of Yoda1 and IL6 over 24-hrs resulted in an increased release of IL2 (1.3-fold, $p=0.006$) and IL1β (1.2-fold, $p=0.039$).

DISCUSSION: This study highlights the importance of the Piezo1 mechanosensitive channel in linking mechanical and inflammatory pathways in osteocytes. The Piezo1 channel opens in response to mechanical stimuli, transducing mechanical signals into an inflammatory cascade in the cell leading to tissue inflammation and playing a vital role in the occurrence and progression of chronic inflammatory diseases [9]. Yoda1 acts as a molecular wedge and in the absence of load induces conformational changes in the plasma membrane leading to opening of the Piezo1 channel. In the current study, Yoda1 mediated an increase in IL6 signaling, a cytokine that drives inflammation and bone resorption and has been implicated in the onset of post-traumatic osteoarthritis (PTOA).

SIGNIFICANCE/CLINICAL RELEVANCE: Our study demonstrates that PIEZO1 is an important mechanosensor in osteocytes, supporting previous studies [2]. This provides a direct link between mechanical activation of Piezo1 and inflammation, which may contribute to mechanically induced joint degeneration in diseases such as osteoarthritis. Understanding the role of Piezo1 in osteocyte signaling is important since mechanisms underlying abnormal joint mechanics are poorly understood despite it being the major risk factor for developing osteoarthritis.

REFERENCES: [1] Vazquez et al. (2014) Front. Endocrinol. 5:208; [2] Gilbert et al. (2022) ORS meeting transact; [3] Gilbert et al. (2018) JOR 36(8):2118; [4] Li et al. (2019) eLife 8:e49631; [5] Morris et al. (2019) Nat Genet. 51(2)258; [6] James et al. (2015) Stem Cell Reports 4(6):1004; [7] Galarza Torre et al. (2018) Sci Rep 8,8981; [8] Syeda et al. (2015) eLife 4:e07369; [9] Liu et al. (2022) Front. Immunol. 13:816149.

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IMAGES AND TABLES:

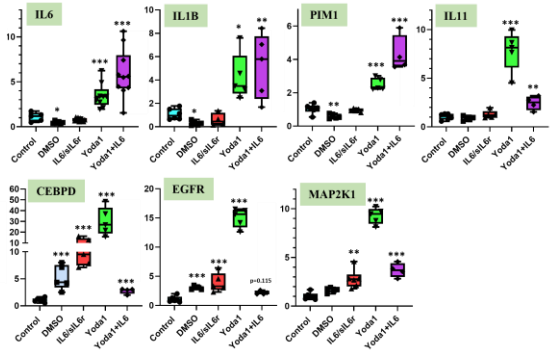


**Figure 1. Validation of PCR arrays.** Y201-derived osteocytes were subjected to DMSO vehicle (0.05%), Yoda1 (5μM), IL6 (5ng/ml)/sIL6r (40ng/ml), or Yoda1+IL6 for 24-hrs, and the expression levels of genes identified by PCR array measured by RT-qPCR; data was normalized to the housekeeping gene, 18S (One way ANOVA and Tukey post-hoc tests n=4-10).